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(54) Use of yeast cell debris products.

(57) Colouring agents are disclosed comprising glucan-containing yeast cell ghosts which comprise a proportion of substantially intact yeast cell walls and a colour source. The colour source may be naturally occurring and may, for example, be provided by turmeric or annatto. A process for preparing the colouring agents comprises providing an aqueous mixture of colour source and yeast cell ghosts, separating insoluble solids from the mixture and drying the insoluble solids. A process for preparing the yeast cell ghosts for use in the invention comprises the extraction of yeast debris with acid, treatment with alkali, separation of whole cells, bleaching and, optionally, acidifying the bleached material.

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This invention relates to the use of yeast cell debris products in foodstuffs, cosmetics and pharmaceuticals.

Yeast extract is commercially produced on a large scale by lysis (e.g., hydrolysis, autolysis or plasmolysis) of baker's yeast or brewer's yeast in suitable form or of other fermentation yeasts (e.g., from gasohol production), which results in soluble material and material which is rich in virtually intact cell wall bodies. The latter material is normally removed from the soluble material by centrifugation. The lysis inevitably results in some disruption of the cell walls, such that there is a substantial proportion of virtually intact cell wall bodies, with at least one zone of discontinuity in the cell wall surface region (that is, holes have resulted in the relevant cell walls). The material containing cell wall bodies (known as yeast debris, or colloquially as yeast ref), which has a dark brown colour, an unpleasant odour and rapidly putrefies, contains a number of undesirable materials, such as trace elements, colouring agents, hop extracts, tartrates, microorganisms; bacteria, protein slime and a large amount of insoluble components such as yeast cell wall bodies, as well as a certain amount of unlysed whole cells; such yeast debris is normally discarded. The soluble material from which the debris has been separated is normally used for the extraction of useful materials, such as yeast extract.

PCT/GB91/01819 discloses a process of treating yeast debris which produces a purified form of yeast ghosts or shells having substantially intact cell walls (that is, retaining the in vivo morphology of yeast cell walls in yeast debris), but without the yeast cell contents. That is, the yeast ghosts correspond in morphology to that of lysed material (the yeast debris, or ref) and not that of the whole yeast cells; the yeast ghosts comprise essentially yeast beta-glucan.

Yeast beta-glucans are, of course, well known. US 4,810,646 discloses a method of producing yeast beta-glucans, which comprises separating growing Saccharomyces cerevisiae yeast from its growth medium, subjecting the intact whole yeast cells to alkaline digestion to solubilise the protein portion of the cell, and treating the insoluble glucan with acetic acid to alter the beta (1,6) linkages. The resulting whole glucan particles are described in US 4,962,094 as being suitable for use as dietary additives and are said to substantially retain the in vivo glucan three dimensional molecular structure of the yeast cells from which they are derived. However, the cell walls are effectively destroyed in the method described. In PCT/GB91/01819, the yeast ghosts or shells are obtained from yeast debris without destruction of the cell wall structure.

The term yeast cell ghosts, as used herein, covers yeast ghosts or shells having a proportion of yeast cell wall which is substantially intact.

The present invention is based on applications of the yeast cell ghosts described in PCT/GB91/01819.

It has been found that yeast cell ghosts may be used to produce colouring agents which are insoluble in aqueous media. The colouring agents may be produced from natural substances thus avoiding the use of artificial carriers and/or colourings. The colouring agents may be used to impart colour to a variety of materials including foodstuffs, cosmetics and pharmaceuticals.

Accordingly, the present invention provides a colouring agent comprising glucan-containing yeast cell ghosts which comprise a proportion of substantially intact yeast cell walls and at least one colour source. The colour source is selected to give the desired colour in the colouring agent and in any product in which the colouring agent is used and may be any dye or pigment. For uses such as in foodstuffs, pharmaceuticals and cosmetics, the colouring agents must be acceptable for food and/or pharmaceutical use. Preferably, the colour sources are either naturally occurring or are provided by naturally occurring products such as turmeric and annatto. The colour sources may be partly or fully purified from their natural source or used without purification.

The present invention also provides a process for producing a colouring agent comprising the steps of:

(i) providing an aqueous mixture of at least one colour source and yeast cell ghosts which comprise a proportion of substantially intact yeast cell walls;

(ii) separating insoluble solids from the mixture; and

(iii) drying the insoluble solids.

The aqueous mixture may be treated to optimise the formation of the colouring agent e.g., by adjusting the pH of the mixture.

Preferably, the aqueous mixture is formed by first adding at least one colour source and subsequently adding yeast cell ghosts to water or another suitable aqueous solution. The mixture may be stirred for at least 30 seconds, preferably for 30 seconds to 5 minutes (e.g., 1 minute). The insoluble solids may be separated from the aqueous mixture in any one of a number of ways well known to those skilled in the art e.g., by centrifugation. The insoluble solids are preferably washed before drying preferably with water. The insoluble solids may be dried in a number of ways e.g., by freeze drying.

The colouring agents of the present invention may be used to provide colour in foodstuffs, cosmetics and pharm-aceutical compositions.

Yeast cell ghosts for use in the present invention may be produced by the process of treating yeast debris having a solids content not exceeding 20% by weight which is disclosed in PCT/GB91/01819 and which comprises:

(a) extracting said debris with a food grade alkaline salt;

(b) separating whole cells from the extracted debris so as to leave a material rich in disrupted but otherwise intact cell walls;

(c) treatment of the latter material with an alkaline extraction agent;

(d) bleaching said material with a bleaching agent or food grade oxidising/reducing agent (such as ascorbic acid) either before or after said separation step; and

(e) lowering the pH of said bleached material using a food grade acid (such as citric acid, orthophosphoric acid, dilute hydrochloric acid or dilute sulphuric acid).

Typical food grade alkaline salts for use in step (a) include sodium, calcium or potassium bicarbonate, or sodium, calcium or potassium carbonate; sodium bicarbonate is most preferred. The yeast debris typically has a solids content of about 2 to 12%, such as 4 to 8% (generally about 5%) by weight and a viscosity in the range of about 5-15 cP (5% aqueous suspension). The debris is generally extracted in step (a) of the process according to the invention with the alkaline salt, generally at substantially ambient temperatures for approximately one hour. The alkaline salt (which, as previously mentioned, is preferably sodium bicarbonate) is preferably used in an amount of up to 2.5% (preferably about 1%) weight, based on the total volume of yeast debris (liquids and solids) and preferably such that the resulting extracted mix has a pH in the range 8 to 12, more preferably about 8 to 9.

The separation of whole cells from the extracted debris so as to produce material rich in disrupted cell walls is generally carried out by mechanical methods, of which centrifugation is preferred. The centrifugation is typically operated at about 5,000 rpm for differential centrifugation or about 2,500 rpm for static centrifugation. The use of a bicarbonate in stage (a) has been found to assist the separation stage (possibly because of gas evolution which serves to make the yeast cell ghosts lighter).

Following separation, the material is treated with an alkaline extraction and extraction agent such as potassium hydroxide, sodium hydroxide or calcium hydroxide. This treatment with an alkaline extraction agent (which is similar to the process known as mercerisation) typically involves treatment in an alkaline solution having a pH of 8 to 14, preferably about 12 to 12.5. The treatment assists in removing coloured products, dissolving unwanted materials such as protein, opening up the structure of the cell walls and facilitating the bleaching step. The mixture is then preferably heated to a temperature in the range of about 65° to 85°C for at least one hour. If the final product is required to have a pale cream colour, it is preferred that the alkali used comprises potassium or sodium hydroxide; however if the final product is required to have a white colour it is preferred that the alkali used comprises calcium hydroxide.

The bleaching stage is preferably carried out using hydrogen peroxide or a food-grade oxidising/ reducing agent (such as ascorbic acid) when the product is to be used for food purposes; the bleaching is preferably carried out such that the bleached material is pale cream or white in colour. The bleaching stage is preferably carried out in a reactor and the quantity of material rich in disrupted cell walls introduced into the reactor is preferably monitored such that the material occupies not more than about half of the reactor volume. This is because the bleaching stage typically involves foaming which causes a substantial increase in the volume of the material being treated. Preferably the foaming is substantially controlled using a foam breaking paddle and can be substantially lessened by the addition of anti-foaming agents.

Optionally the bleached material may be treated with a food grade acid (typically hydrochloric or orthophosphoric acid), centrifuged and treated with lecithin. The material may then be further centrifuged prior to drying. Treatment of the bleached material with lecithin is advantageous because lecithin helps to mask any residual flavour or odour pertaining to the yeast debris.

The lowering of the pH with the food grade acid may be carried out by washing the material being centrifuged, or may be carried out subsequently. The pH is generally lowered to a pH of 5 to 6, either in a single stage, or by lowering initially to a value from about 6 to 7.5 (such as about 7.0) and at a later stage to pH about 5 to 6.

Yeast cell ghosts may also be produced by the improved process of the present invention which provides a process for producing yeast cell ghosts which effects greater de-flavouring and de-colouring than previous processes.

Accordingly, the invention provides a process for preparing yeast cell ghosts which comprise at least a proportion of substantially intact yeast cell walls which comprises the steps of:

(i) extracting yeast debris having a solids content not exceeding 20% by weight with acid;

(ii) treating the extracted debris with an alkali;

(iii) separating whole cells from the treated mixture so as to leave a material rich in disrupted but otherwise intact cell walls;

(iv) bleaching said material with a bleaching agent or food grade oxidising/reducing agents (such as ascorbic acid) after said separation step; and, optionally,

(v) lowering the pH of said bleached material using a food grade acid (such as citric acid, dilute hydrochloric acid or dilute sulphuric acid)

The yeast debris used in step (i) typically has a solids content of about 2 to 10%, such as 4 to 8% (e.g., about 5%) by weight and a viscosity in the range of about 5-15 cP (5% aqueous suspension). The debris is generally extracted at 50-100°C (e.g., 60-70°C)

for 30 minutes to 2 hours (e.g., 1 hour). Many acids may be used in the process (e.g., hydrochloric sulphuric, phosphoric and citric acids) either alone or as a mixture of two or more acids. The acid is present at a concentration effective to carry out step (i). Ascorbic acid is preferably used in an amount of up to 2.5% preferably 0.5 to 1% (e.g., 1%) by weight based on the total volume of yeast debris (liquids and solids) and is preferably added as a solid. Ascorbic acid effects both partial hydrolysis and partial oxidation of the yeast debris.

The alkali treatment in step (ii) may be carried out with an inorganic alkali such as potassium hydroxide, sodium hydroxide or calcium hydroxide. The treatment is typically carried out in an alkaline solution having a pH of 8 to 14, preferably about 12 to 12.5. The yeast debris mixture is preferably diluted to from 1 to 5% (e.g., from 2 to 3%) w/v prior to treatment. The mixture is heated to 65° to 85°C for 30 minutes to 2 hours (e.g., about 1 hour). Step (ii) is thus complete in a shorter time than the corresponding step in the known process. If the final product is required to have a pale cream colour, it is preferred that the alkali used comprises potassium or sodium hydroxide; however if the final product is required to have a white colour it is preferred that the alkali used comprises calcium hydroxide.

The separation of whole cells from the extracted debris so as to produce material rich in disrupted cell walls (step (iii)) is generally carried out by mechanical methods, of which centrifugation is preferred. The centrifuge is typically operated at about 5,000 rpm for differential centrifugation or about 2,500 rpm for static centrifugation. Preferably the alkalinity is reduced (but preferably not below pH 9.5) prior to centrifugation.

The bleaching stage (step (iv)) is preferably carried out using hydrogen peroxide or a food-grade oxidising/reducing agent (such as ascorbic acid) when the product is to be used for food purposes; the bleaching is preferably carried out such that the bleached material is pale cream or white in colour, which is advantageous when the resulting product is intended for use as a food grade material such as functional fibre. The bleaching stage is preferably carried out in a reactor and the quantity of material rich in disrupted cell walls introduced into the reactor is preferably monitored such that the material occupies not more than about half of the reactor volume. This is because the bleaching stage typically involves foaming which causes a substantially increase in the volume of the material being treated. Preferably for foaming is substantially controlled using a foam breaking paddle and can be substantially lessened by the addition of anti-foaming agents.

The bleached product may be further treated by neutralisation with ascorbic acid (preferably as a concentrated aqueous solution), centrifugation and washing.

The process of the invention provides a yeast cell ghost product having less flavour and a lighter colour than previous processes.

Example 1

Brewer's yeast was subjected to autolysis, for breaking up of the cell membranes. The lysed product was then separated by centrifugation to produce yeast debris the fraction containing the cell bodies having a viscosity of 10 cP (5% aqueous solution).

The yeast debris was then treated with sodium bicarbonate so as to produce an extracted mix having a pH of 8.5. The mix was then stirred for about one hour at room temperature, and centrifuged for further separation.

The centrifuged material resulted in two streams, namely cell wall rich material and undegraded cells. The cell wall rich material was resuspended in 2.5% sodium hydroxide and then 40% sodium hydroxide added so as to adjust the pH of the material to 12.5. The cell wall rich material was then indirectly heated on a water bath to about 65°C for about 1 hour and then bleached by treatment with hydrogen peroxide, for about one hour with mixing. Concentrated hydrochloric acid was then added to the bleached material so as to achieve a pH of 7.0; the material was further centrifuged and the pH further lowered to 5.0. The resulting product was substantially free of any whole yeast cells and predominantly comprised yeast ghosts or shells having substantially uncollapsed walls. The yeast ghosts contained a lower quantity of yeast cell contents relative to the whole cells of the debris.

Water (200 ml) was acidified to pH 2 with dilute hydrochloric acid and turmeric (0.3 g) added to the resulting solution. The yeast cell ghosts (8.0 g) were added to the solution and the mixture stirred for 1 minute, centrifuged, washed twice with water and freeze-dried to give a stable canary yellow lake.

Example 2

Water (200 ml) was acidified to pH 6 with dilute hydrochloric acid and annatto (0.3 g) added to the resulting solution. Yeast cell ghosts (8.0 g) as prepared in Example 1 were added to the solution and the mixture stirred for 1 minute, centrifuged, washed twice with water and freeze-dried to give a stable orange lake.

Claims

1 A colouring agent comprising glucan-containing yeast cell ghosts which comprise a proportion of substantially intact yeast cell walls and at least one colour

source.

2 A colouring agent as claimed in claim 1, wherein the colour source is naturally occurring.

3 A colouring agent as claimed in claim 2, wherein the colour source is provided by turmeric or annatto.

4 A process for producing a colouring agent comprising the steps of:

(i) providing an aqueous mixture of at least one colour source and yeast cell ghosts which comprise a proportion of substantially intact yeast cell walls;

(ii) separating insoluble solids from the mixture; and

(iii) drying the insoluble solids.

5 A process as claimed in claim 4, wherein the pH of the aqueous mixture is adjusted to optimise the formation of the colouring agent.

6 A process as claimed in claim 4 or claim 5, wherein the aqueous mixture is stirred for at least 30 seconds.

7 A process as claimed in claims 4 to 6, wherein the aqueous mixture is formed by first adding at least one colour source and subsequently adding yeast cell ghosts to water.

8 A process as claimed in claims 4 to 7, wherein the insoluble solids are separated from the mixture by centrifugation.

9 A process as claimed in claims 4 to 8, wherein the insoluble solids are washed before drying.

10 A process as claimed in claims 4 to 9, wherein the insoluble solids are freeze dried.

11 A pharmaceutical composition comprising the colouring agent of claims 1 to 3 and a pharmaceutically active substance.

12 A process for preparing yeast cell ghosts which comprise at least a proportion of substantially intact yeast cell walls which comprises the steps of:

(i) extracting yeast debris having a solids content not exceeding 20% by weight with acid;

(ii) treating the extracted debris with an alkali;

(iii) separating whole cells from the treated mixture so as to leave a material rich in disrupted but otherwise intact cell walls;

(iv) bleaching said material with a bleaching agent or food grade oxidising/reducing agents after said separation step; and, optionally,

(v) lowering the pH of said bleached material using a food grade acid.

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(54) **Use of yeast cell debris products.**

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EUROPEAN SEARCH REPORT

Application Number
EP 93 30 2833

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.5)
Y	EP-A-0 474 347 (QUEST INT. B.V.) * page 5, line 30 - line 55; claims 4,6-9 * ---	1-12	C12N1/16 C09B61/00 A61K9/50 C12N1/06 A23L1/00
D,Y	US-A-4 810 646 (SPIROS JAMAS ET AL.) * the whole document * ---	1-12	
Y	US-A-4 574 086 (J.R.SHACKELFORD) * abstract; example 7 * ---	3,7	
Y	DATABASE WPI Section Ch, Week 7949, Derwent Publications Ltd., London, GB; Class B04, AN 79-88295B & JP-A-54 138 115 (KIRIN BREWERY KK) 26 October 1979 * abstract * ---	12	
A	DATABASE WPI Section Ch, Week 7917, Derwent Publications Ltd., London, GB; Class D13, AN 79-32367B & JP-A-54 035 249 (IDEMITSU INDUSTRIES KK) 15 March 1979 * abstract * -----	11	

TECHNICAL FIELDS SEARCHED (Int.Cl.5)

C12N

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
BERLIN	8 April 1994	Gurdjian, D

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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